

## Lipofectamine

1. Transfection is done after 24 hr incubation of the plated cells at 37°C.
2. For each transfection sample, prepare complexes as follows:
  - a. Dilute DNA (1.0 µg) in 100 µl of RPMI 1% Glu 1% PS **without serum**. Mix gently.
  - b. Mix Lipofectamine® 2000 gently before use, then add 4 µl to 96 µl of RPMI 1% Glu 1% PS **without serum**. Incubate for 5 minutes at room temperature. Proceed to Step c within 25 minutes.
  - c. After the 5 minute incubation, combine the diluted DNA with diluted Lipofectamine® 2000 (total volume = 1 ml). Mix gently and incubate for 20 minutes at room temperature (solution may appear cloudy). Note: Complexes are stable for 6 hours at room temperature.
3. Add the 200 µl of complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.
4. Incubate for 3 hours at 37°C.
5. Change medium to RPMI 1% Glu 1% PS **with 10% FBS**.
6. Incubate cells at 37°C in a CO2 incubator for 18-48 hours prior to testing for transgene expression.